REMARKS

Claims 16-21 and 28-44 are pending following entry of the amendment presented above. Claims 22-27 have been cancelled, Claim 16 amended, and Claims 38-44 added. The marked up versions of the specification and claim amendments are attached hereto and are captioned "Version with Markings to Show Changes Made to the Specification" and "Version with Markings to Show Changes Made to the Claims," respectively.

The issues raised in the outstanding Office Action and support for the new claims will be addressed below.

I. Information Disclosure Statement.

The Office Action states that the International Search Report does not constitute a publication under USPTO guidelines. This document was submitted in the interest of full disclosure in the event that the Examiner wished to review the content of the International Search Report when considering the references cited therein.

II. Triton X-100.

The specification stands objected to on the basis that the term "Triton X-100" is a trademark and should be capitalized wherever it appears and should be accompanied by generic terminology. Applicants have amended the specification as requested by the Examiner to address this objection. In addition, the term "Triton X-100" has been capitalized and accompanied by the generic term in the claims as well.

III. Indefiniteness.

Claims 16-37 stand rejected under 35 U.S.C. § 112, second paragraph, as indefinite. Claims 22-27 have been cancelled. These rejections will be addressed below with respect to the remaining claims.

First, the Office Action states that "it is unclear how the term 'amplification motif' in claim 32 is defined in the specification." (Office Action,

page 3, second paragraph). This rejection has been addressed in the parent application.

As discussed therein, Claims 32-37 recite steps for synthesizing dsDNA from ssRNA. This is illustrated in Figure 7. In the cDNA synthesis step, primers are used that will incorporate specific sequences into the single stranded cDNA, whereby said specific sequences are used as recognition sites for PCR primers in the subsequent PCR reaction (*e.g.*, Claim 36) using the cDNA as a template. The idea is that this method will provide a way to amplify a nucleic acid by PCR while the original sequence to be amplified is unknown (thus, no specific primers can be made). This can be seen from page 17, last paragraph, where it is stated that: "The method described here is useful in isolating and characterizing unknown sequences present in clinical samples (e.g. viral sequences) or for the amplification of cDNAs from transcripts without having any sequence data."

The amplification motif and sequencing motif are sequences that are built into the primers used in cDNA synthesis with the aim to make them part of the cDNA product. The rest of the sequence of the cDNA product is unknown. With the aid of these known sequences that have been made part of the cDNA, follow-up reactions can be carried out. The "amplification motif" that is part of the primers used in the cDNA synthesis is a sequence that will be recognized by the PCR primers used in the PCR reaction following cDNA synthesis. This can be seen from pages 13 and 14 of the specification. At the bottom of page 13, for example, two oligonucleotides are shown that each have an underlined sequence. This underlined sequence is the "amplification motif," and it is the same as the sequence in the PCR primer RB 8 shown on the same page. From Figure 7, it can be seen that when the synthesis of double stranded cDNA is completed, both strands of this double stranded cDNA will have the "amplification motif" as well as its complement incorporated into the sequence. Since the PCR primer will anneal to both strands of cDNA, it can be used to carry out a PCR reaction. The specific

sequence of the "amplification motif" is not critical, as explained at page 6, lines 15-21.

Accordingly, Applicants submit that the claim term "amplification motif" as recited in the claims is sufficiently clear.

Second, the Office Action states that the language "a mixture of both" in Claim 16 is vague and indefinite. Claim 16 has been amended to clarify that the mixture contains both single-stranded and double-stranded nucleic acids. Applicants note that this is not a narrowing amendment.

In view of the foregoing, Applicants submit that the claims satisfy the requirements of 35 U.S.C. § 112, second paragraph, and respectfully request that the rejections on this basis be withdrawn.

IV. Rejections under § 103 (a).

Claims 16-21, 25-26 and 29-31 stand rejected under 35 U.S.C. § 103(a) for obviousness over WO 95/04140 (Boom et al.). Claims 32-37 stand rejected under 35 U.S.C. § 103(a) for obviousness over U.S. 5,043, 272 (Hartley et al.) in view of U.S. 4,965,188 (Mullis et al.) and Sambrook et al. These rejections are respectfully traversed below.

A. Legal Standards for Obviousness.

The Patent Office has the initial burden under §103 to establish a *prima facie* case of obviousness. *In re Fine*, 837 F.2d 1071 , 5 USPQ2d 1596, 1598 (Fed. Cir. 1988). The Applicants respectfully note that in order to establish a *prima facie* case of obviousness, three basic criteria must be met. First, there must be some suggestion or motivation, either in the references themselves or in the knowledge generally available to one of ordinary skill in the art, to modify the reference or to combine reference teachings in order to arrive at the claimed invention. Second, there must be a reasonable expectation of success. Third, the prior art reference (or references when combined) must teach or suggest all of the claim limitations (MPEP § 706.02(j)). The teaching or suggestion to make the claimed combination and the reasonable expectation of success must both

be found in the prior art, and not based on applicant's disclosure. *In re Vaeck*, 947 F.2d 488, 20 USPQ2d 1438 (Fed. Cir. 1991).

The Federal Circuit has articulated the following legal test for obviousness: "The consistent criterion for determination of obviousness is whether the prior art would have suggested to one of ordinary skill in the art that this process should be carried out and would have a reasonable likelihood of success, viewed in the light of the prior art. . . . Both the suggestion and the expectation of success must be founded in the prior art, not in the applicant's disclosure." *In re Dow Chemical*, 5 USPQ2d 1529, 1531 (Fed. Cir. 1988) (emphasis added). Obviousness cannot be established by combining the teachings of the prior art to produce the claimed invention, absent some teaching, suggestion, or incentive supporting the combination. *In re Geiger*, 2 USPQ2d 1276 (CAFC 1987). The mere fact that references can be combined does not render the combination obvious unless the prior art also suggests the desirability of the combination. *In re Fritch*, 23 USPQ 2d 1780 (CAFC 1992). The Court of Appeals for the Federal Circuit has addressed this issue and has stated that "[t]he mere fact that the prior art could be so modified would not have made the modification obvious unless the prior art suggested the desirability of the modification." *In re Gordon*, 221 USPQ, 1125, 1127 (Fed. Cir. 1985) (emphasis added).

The Applicants respectfully contend that the Patent Office has failed to establish a *prima facie case* of obviousness in the present case.

B. The Claims are Patentable over Boom et al.

Claims 16-21, 25, 26 and 29-31 stand rejected under 35 U.S.C. § 103(a) for obviousness over Boom et al. Claims 25 and 26 have been cancelled. This rejection is respectfully traversed below with respect to Claims 16-21 and 29-31.

The Office Action states that Protocol B in Boom et al. describes a method of isolating dsDNA, and further that Protocol Y may be used for

"simultaneous purification" of dsDNA and ssDNA. The Office Action further states:

> Boom et al. do not disclose using the second liquid composition for purifying a single stranded nucleic acid as claimed. However, Boom et al. do disclose that the protocol is used for isolating single stranded nucleic acid sequence (See pg. 15, lines 1-22). One of ordinary skill in the art at the time of the instant invention would have been motivated to apply a second time isolation of a single nucleic acid from the supernatant containing single stranded nucleic acid with a reasonable expectation of success because repeating purification steps for a reasonable expectation of success is a routine practice for purification in the art. In fact, the protocol Y of Boom is used for isolating a single stranded nucleic acid (See pg. 15, lines 1-22).

(Office Action, paragraphs spanning pages 4-5, emphasis added).

Applicants respectfully submit that the basis of the present rejection is unclear. In particular, it is confusing as to why the Examiner is maintaining the position that protocol Y of Boom et al. teaches a method for isolating ssDNA, when the reference itself quite explicitly states that it does not. Rather, it is taught that the protocol Y of Boom et al. may be used to isolate nucleic acids from other materials, but may not be used to separate ssDNA from dsDNA (*i.e.*, this protocol cannot distinguish ssDNA from dsDNA). If the present rejection is repeated, it is respectfully requested that the Examiner clarify the present rejection so that Applicants may respond more fully thereto and so as to create a more complete record in the event an appeal is filed.

The present inventors have found that conditions may be manipulated so that dsDNA and ssDNA may be sequentially isolated from a composition containing a mixture of both dsDNA and ssDNA. There is no suggestion in Boom et al. that dsDNA and ssDNA may be isolated away from each other by controlling the composition of the isolation medium. Indeed, protocol Y of Boom et al. only teaches a method for simultaneously isolating dsDNA and ssDNA.

The Office Action states that there would be a "reasonable expectation of success because repeating purification steps for a reasonable expectation of success is routine practice." (Office Action, page 5, lines 3-4). However, as Applicants have previously stated, repeating protocol Y of Boom et al. would not result in separation of dsDNA from ssDNA, but would only result in multiple steps yielding both dsDNA and ssDNA.

Moreover, it was not appreciated prior to the work described in the present application that ssDNA would not bind to a solid phase in the GEDTA medium described in protocol B of Boom et al. In fact, it would not have been expected that significant nucleic acids would be left in the supernatant produced in protocol B, and it certainly would not have been expected that it would preferentially contain ssDNA. As stated by the present application at page 3 (lines 33-35; emphasis added): "By serendipity we found that ss-nucleic acid did not bind to silica particles or diatomaceous earth in the presence of buffer L11 [GEDTA] (see examples), whereas ds nucleic acid did." Thus, it would not have been obvious to one of ordinary skill in the art from the teachings of Boom et al. that dsDNA would bind preferentially to a solid phase in GEDTA. Claim 16 has been amended to recite that "the double stranded nucleic acid preferentially binds to the solid phase" during the first contacting step to more fully recite this feature of the present invention and so as to further distinguish the presently claimed subject matter from Boom et al.

Thus, the outstanding rejection is legally insufficient to support a *prima facie* case of obviousness. Boom et al. would not have provided any motivation or suggestion to one of ordinary skill in the art at the time of invention to carry out a method of sequentially isolating dsDNA from ssDNA as presently claimed. Accordingly, Applicants respectfully request that the outstanding obviousness rejection be withdrawn.

C. The Claims are Patentable over Hartley et al. in view of Mullis et al. and Sambrook et al.

Claims 32-37 stand rejected under 35 U.S.C. § 103(a) as unpatentable for obviousness over U.S. 5,043,272 (Hartley et al.) in view of U.S. 4,965,188 (Mullis et al.) and Sambrook et al. This rejection is respectfully traversed below.

As an initial matter, Applicants submit that the cited references are not properly combined. As discussed above, a *prima facie* case of obviousness requires that the references themselves suggest the combination, as well as the desirability of making such a combination. In the instant case, there is absolutely no suggestion or motivation in the references themselves to make the combination.

Moreover, even if the references were properly combined, they fail to disclose or suggest the features of the present invention. The primary reference, by Hartley et al., merely discloses a method for achieving a single round of amplification using random primers. The random primers of Hartley et al. are quite distinct from the primers of the present invention.

Claim 32 recites a primer having both a "random hybridization sequence and amplification motif." As described in the specification and shown in Figure 7, the amplification motif is a specific sequence that may be utilized in further amplification steps (*e.g.*, Claim 36). Hartley et al. fails to provide any teachings or suggestion whatsoever for a primer having a random hybridization sequence and an amplification motif according to the present invention.

Claim 32 further recites a second strand synthesis step to produce dsDNA using a second primer having a random hybridization sequence and amplification motif. Again, Hartley fails to suggest the recited primer having a random hybridization sequence and an amplification motif.

The deficiencies of Hartley et al. are not remedied by Mullis et al. or Sambrook et al. Mullis et al. only discloses PCR methods. Sambrook et al. merely indicates that avian reverse transcriptase has RNase H activity.

Thus, the cited references, taken alone or in any combination, fail to suggest the presently recited methods of producing a double-stranded cDNA utilizing a first and second primer, each primer comprising a random hybridization sequence and an amplification motif.

With particular respect to Claim 36, this claim further recites the step of amplifying the double-stranded cDNA of Claim 32 "using at least one primer that specifically anneals to the amplification motif." As noted above, none of the cited references provide any suggestion regarding the inventive amplification motif. Clearly, it follows that there is absolutely no suggestion or motivation whatsoever in the cited references to amplify a cDNA using a primer that specifically anneals to the amplification motif.

Accordingly, for the reasons set forth in detail above, Applicants submit that the subject matter of Claims 32-37 is novel and unobvious over the combination of Hartley et al., Mullis et al. and Sambrook et al., and respectfully request that the outstanding rejection under § 103(a) be withdrawn.

D. <u>New Claims 38-44</u>.

New Claims 38-43 are a rewriting of canceled Claims 22-27, with the subject matter of Claim 22 placed into independent form in new Claim 38. The Office Action indicated that Claims 22-24 and 27 would be allowable if rewritten in independent form to include the limitations of the base claim and any intervening claims and if the rejections under § 112, second paragraph, were addressed. The indefiniteness rejections raised in the outstanding Office Action were addressed above. Accordingly, new Claims 38-40 and 43 are also free of the outstanding art rejections. Applicants further submit that Claims 25 and 26 (and new Claims 41 and 42), which ultimately depend from Claim 22 (Claim 38), are also free of the art as they depend from an allowable base claim.

With respect to new Claim 44, this claim is the same as Claim 16 except that it further recites that the second liquid comprises "divalent positive

ions." This recitation is supported throughout the specification, which teaches that divalent positive ions restore binding of ssDNA to a solid phase (*see, e.g.*, sentence spanning pages 3-4). Applicants submit that Boom et al. does not disclose a liquid comprising a "chaotropic agent and divalent positive ions" as recited by Claim 44, and, thus, further submit that this claim is free of the outstanding rejection over Boom et al.

In light of the foregoing discussion, it is submitted that the subject matter of new Claims 38-44 is free of the art of record.

V. Sequence Listing.

The specification stands objected to on the basis that the disclosure contains nucleic acid sequences encompassed by 37 C.F.R. § 1.821(a)(1) and §1.821(a)(2), which are not accompanied by the appropriate sequence identifiers. Applicants have amended page 13 of the specification herein to add the appropriate sequence identifiers, thereby addressing the present objection to the specification.

The Office Action does not indicate that an additional paper or computer readable copy of the sequence listing is required, but will do so at the Examiner's request.

VI. Conclusion.

The points and concerns raised by the Examiner in the outstanding Office Action having been addressed in full, it is respectfully submitted that this application is in condition for allowance, which action is respectfully requested. Should the Examiner have any remaining concerns, it is requested that the Examiner contact the undersigned attorney to expedite the prosecution of this application to allowance.

Respectfully submitted,

Karen Magri

Karen A. Magri
Registration No. 41,965

Customer Number:

20792
PATENT TRADEMARK OFFICE

CERTIFICATE OF MAILING

I hereby certify that this correspondence is being deposited with the United States Postal Service as first class mail in an envelope addressed to: Commissioner for Patents, Washington, DC 20231, on June 19, 2002.

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Version with Markings To Show Changes Made to the Specification

Please amend the paragraph at page 7, lines 12-17, as follows:

-- EDTA (Titriplex) and MgCl2.6H20 were obtained from Merck (Darmstadt, Germany). Tris was obtained from Boehringer (Mannheim, Germany). The preparation of size-fractionated silica particles (silica coarse, SC) and diatom suspension has been described (11). TRITON X-100™ (polyethoxylated p-isooctyl-phenol) [Triton X-100] was from Packard (Packard Instrument Co., Inc., Downers Grove, Ill).--

Please amend the paragraph at page 7, lines 20-32, as follows:

--The lysis/binding buffer L6, washing buffer L2, and TE (10mM Tris.HCl, 1 mM EDTA; pH=8.0) was made by dissolving 37.2 g EDTA (Merck, Germany) and 4.4 g NaOH (Merck, Germany) in aqua in a total volume of 500 ml. Lysis/binding buffer L11 was made by dissolving 120 g of GuSCN in 100 ml 0.2M EDTA (pH=8.0). Binding buffer L10 was prepared by dissolving 120 g GusCN in 100 ml 0.35M TRIS.HCl (pH 6.4); subsequently 22 ml 0.2M EDTA (pH 8.0) and 0.1 g TRITON X-100™ (polyethoxylated p-isooctyl-phenol) [Triton X-100] were added and the solution was homogenized; finally 11 g of solid $MgCl_2.6H_20$ was added. The final concentration of $MgCl_2$ in L10 is about 0.25M. L10 is stable for at least 1 month when stored at ambient temperature in the dark.--

Please amend the paragraph at page 12, lines 28-33, as follows:

-- EDTA, KCl, $MgCl_2.6H_2O$, NaCl and tri-Sodium citrate dihydrate were obtained from Merck (Darmstadt, Germany). TRIS and BSA were obtained from Boehringer (Mannheim, Germany). TRITON X-100™ (polyethoxylated p-isooctyl-phenol) [Triton X-100] was obtained from Packard (Packard Instruments Co., Inc., Downers, Ill, USA). Sodium Dodecylsulfate (SDS) was obtained from Serva (Heidelberg, Germany).--

Please amend the paragraph at page 13, lines 17-18, as follows:

-- The 10 x reverse transcription buffer (CMB1) consists of 100 mM Tris.HCl (pH 8.5), 500 mM KCl and 1% TRITON X-100™ (polyethoxylated p-isooctyl-phenol) [Triton X-100].--

Please amend the paragraph at page 13, lines 28-38, as follows:

--The first strand primer **TAG 20:**

5'GACAGAATGCCGAAATGACCCCNNNNNG3' (SEQ ID NO:1)

The second strand primer **TAG 7**:

5'DIG- GACAGAATGCCGAAATGANNNNNG3' (SEQ ID NO:2)

The PCR primer RB 8:

5' GACAGAATGCCGAAATGA3' (SEQ ID NO:3) --

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Version with Markings to Show Changes Made to the Claims

Please amend Claim 16 as follows:

16. (Amended) A method for separating single stranded nucleic acid from double stranded nucleic acid, comprising the steps of:

contacting a mixture [of] comprising both single stranded nucleic acid and double stranded nucleic acid with a first liquid comprising a chaotropic agent and a nucleic acid binding solid phase, wherein the first liquid has a composition such that the double stranded nucleic acid preferentially binds to the solid phase;

separating the solid phase from a supernatant containing the single stranded nucleic acid; and

treating the supernatant with a second liquid comprising a chaotropic agent and a second nucleic acid binding solid phase, wherein the second liquid has a composition such that the resulting mixture of supernatant and second liquid allows for binding of the single stranded nucleic acid material to the second solid phase, whereby the single stranded nucleic acid is isolated.

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